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Development of sonosensitive Poly-(L)-lactic acid nanoparticles

Abstract: Due to serious side effects of traditional chemotherapeutic treatment, novel treatment techniques like targeted drug delivery, which allows a reduction of the overall dosage of drugs, are investigated. It is worth mentioning that at the same time, precise drug delivery offers an increased dosage of chemotherapeutic drugs in the tumorous area employing the EPR effect. Therefore, vehicles smaller than 400 nm can be used to pass the poorly aligned endothelial cells of tumour vessels passively through their fenestrations. In a subsequent step, the chemotherapeutic drugs need to be released. One possibility is an ultrasound-based release via inertial cavitation. Thereby, it is desirable to restrict the drug release to a narrow range. Thus, the cavitation inducing ultrasound wave has to be focused to that region of interest. Ultrasound frequencies of more than 500 kHz enable sufficient focusing, however, inertial cavitation occurs primarily at much lower frequencies. In order to afford inertial cavitation at 500 kHz, either bigger particles in the range of micrometres are needed as cavitation nucleus, which is not possible due to the EPR effect or high acoustic pressure is needed to generate inertial cavitation. Nevertheless, this high pressure is inappropriate for clinical applications due to thermal and mechanical effects on biological tissue.

We have produced Poly-(L)-lactic acid (PLLA) nanoparticles by a solvent evaporation technique that serve as nucleus for inertial cavitation at moderate acoustic pressure of 800 kPa and at high frequencies of 800 kHz after the particles have been freeze-dried. In this contribution, we characterize the designed particles and present the production process. Moreover, we show that these particles enable inertial cavitation at an acoustic pressure and at acoustic frequencies which are commonly used in clinical ultrasound units. We also show that other particles with the same size at the same acoustic parameters do not induce cavitation activity.

Keywords: Cavitation, PLLA, Nanoparticles, Ultrasonic

https://doi.org/10.1515/cdbme-2017-0143

1 Introduction

Although nanoscale technologies still are new, they are already changing the scientific field of medicine. They can be used in imaging, diagnostics and for targeted drug delivery in therapeutics [1]. Targeted drug delivery may give a therapy the opportunity to treat just the desired area and may reduce the whole body of distress of side effects. To achieve this, several different ways can be used such as magnetic drug delivery [2], antibody – drug conjugates [3] and using an passive way, known as the enhanced permeation and retention (EPR) effect. Due to the fact that tumours grow faster than normal tissue, vascularisation happens faster as well which leads to a defective vasculature. This allows particles smaller than 400 nm to accumulate within the tumour [4]. To exploit this consequence of accumulation, drugs can be bound onto the surface of nanoparticles. Tightly focussed release of the drug is the aim in many scientific operations. In this work, it shall be achieved by inertial cavitation using medicinal ultrasound.

Ultrasound frequencies over 500 kHz generally provide good focussing, but with these frequencies only particles in the range of micrometres show cavitation. Therefore, bigger particles or higher pressures are required. However, higher pressure comes with the risk of thermal and mechanical effects on biological tissue.

Microbubbles, such as Optison® with a size less than 10 µm [5] show inertial cavitation below 1 MPa with frequencies over 500 kHz [6]. With the compression and rarefaction of each acoustic wave, the bubble will implode eventually.

The PLLA nanoparticles presented in this work show an equally strong cavitation at 800 kHz and 1.5 MPa with just a

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size of around 110 nm. For nanoparticles to show inertial cavitation, they must act as nucleating agents. Since our nanoparticles are showing cavitation after being freeze-dried and reconstituted, our theory is that they form stable gas pockets on their surfaces due to Laplace pressure.

The presented data compares cavitational activity of our nanoparticles to ultrasound agent Optison® as a positive control and to polystyrene latex beads (300 nm) as negative control.

2 Experimental

Poly-(L)-lactic acid as Resomer L206S and D-(+) Trehalose dehydrate were obtained from Sigma-Aldrich (Taufkirchen, Germany). Poloxamer 188 as LutrolF68 was obtained from BASF (Ludwigshafen, Germany). Dichloromethane and acetone were purchased from Carl Roth (Karlsruhe, Germany). As ultrasound standards, Optison® microbubbles as a gift from GE Healthcare were used and diluted with glucose solution to a 1.7 % (V/V) solution. Both Talcum powder (dispersed to 6.8 % (w/V) in water) and Polystyrene nanospheres (with a nominal mean diameter of 300 nm) were purchased from Sigma-Aldrich (Gillingham, UK), whereas talcum was used as a positive control and nanopspheres as a negative control. Polystyrene latex beads were prepared like the PLLA nanoparticles. Water was double-distilled and then passed through a 0.1 µm membrane filter (polyethersulfone, Sartorius Stedim, Gottingen, Germany). For the filtered PLLA nanoparticles cellulose mixed esters (CME), membrane filters with a pore diameter of 0.8 µm were used (Carl Roth, Karlsruhe, Germany).

2.1 Preparation of nanoparticles

Nanoparticles were produced, using the solvent evaporation technique. A published method [7] was modified by us exploiting two syringes connected with a plastic tube using the Luer locks of both syringes. Two solutions were prepared separately, (i) one aqueous solution with a total volume of 25 mL containing poloxamer 188 (0.27 % w/V) and 15 % w/V trehalose and in addition, (ii) an organic solution with 12 mL acetone and 0.5 mL PLLA Solution (3 % w/V). Both solutions were filled into the syringes and then gently mixed five times. The opalescent mixture was then filled into a 100 mL beaker to stir overnight at 200 rpm and to let the organic solvents evaporate. Afterwards, the nanodispersion was filled to its original volume and passed through a 0.8 µm CME filter. In the following, this preparation will be called NP non-lyophilized. Aliquots of 3 mL were then bottled into freeze-drying vials and transferred to a pre-cooled shelf (Christ Delta, Martin Christ, Osterode am Harz, Germany). The product was held for 48 hours at a chamber pressure of 0.1 mbar and -20 °C for primary drying. For secondary drying, the temperature was increased to 20°C and held for 18 hours.

After lyophilisation, the samples were reconstituted with 3 mL water and either pressed through a 0.8 µm CME filter (in the following called NP filtered) or just reconstituted (NP non-filtered). After reconstitution (and filtering), the sample was tested with ultrasound to measure inertial cavitation.

2.2 Measurement setup

The measurement setup to verify nanoparticle induced cavitation consists of a watertank, a spherical focussed ultrasonic transmitter and a hydrophone (Onda HGL-0200). The transmitter is controlled via rf-amplifier (ENI 3100LA) and an arbitrary function generator (Agilent 33522A), whereat the fundamental frequency of the emitted ultrasound wave is 833 kHz and its peak rarefaction pressure is 1.5 MPa. The ultrasonic transmitter operates in a burst-mode framework (pulse duration 1 ms, repetition frequency 0.5 Hz). Fig. 1 shows the measurement setup.

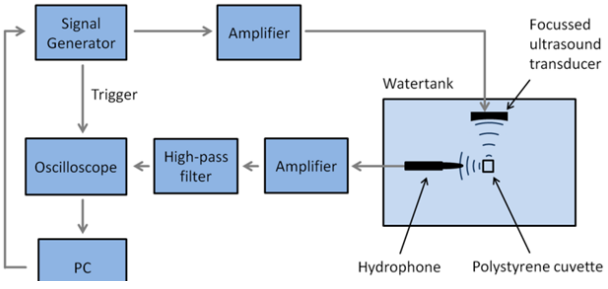

Figure 1: Measurement setup.

A polystyrene cuvette that contains the nanoparticle suspension is located in the focus region of the ultrasound transmitter. As the transmitter, the hydrophone also is aligned to the center locus of the cuvette but is arranged at an angle of 90 degrees. Angular misalignment of hydrophone and ultrasonic transmitter is chosen so that the hydrophone only detects noise signals generated by inertial cavitation taking place in the sample. The hydrophone captures ultrasound waves up to 20 MHz and its output signal is recorded and postprocessed using Matlab.

3 Results

Since high-frequency noise can be seen as an indicator for inertial cavitation, the captured signal is evaluated in the spectral range above the emitting frequency of the ultrasound transmitter. Measurements are performed first on Optison® microbubbles and talcum microparticles, which act as ultrasound standards that are known to be acoustically active at the acoustic parameters described before. In comparison, measurements are also performed with the cuvette containing pure water in order to verify that the captured noise signal follows from particle induced inertial cavitation.

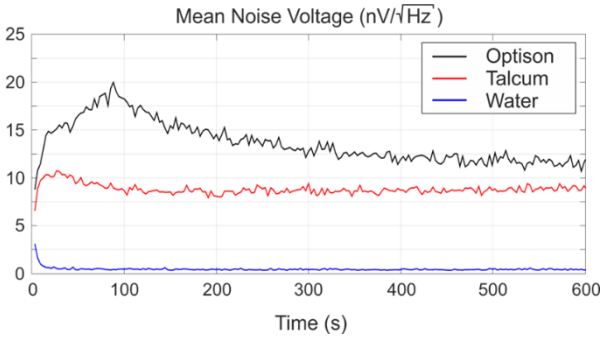

Figure 2: Mean noise voltage between 5 MHz and 6 MHz of the hydrophone output signal with respect to time in the case of the cuvette containing a positive sample (Optison or Talcum) or a negative sample (pure water).

As can be seen from Fig. 2, Optison® and talcum particles lead to high inertial cavitation activity, while as expected pure water does not. The higher acoustic response of Optison® indicates that its microbubbles contain a larger volume of gas than trapped on the surface of the talcum microparticles.

In a second step, the poly-(L)-lactic acid nanoparticles are investigated. To avoid inertial cavitation from cavities that are enclosed within aggregates of particles, the nanoparticle suspension is filtered through a 800 nm pore diameter filter. The particles are investigated in both cases, filtered and non-filtered. Moreover, non-lyophilized nanoparticles and polystyrene nanoparticles are also investigated, which both act as negative samples.

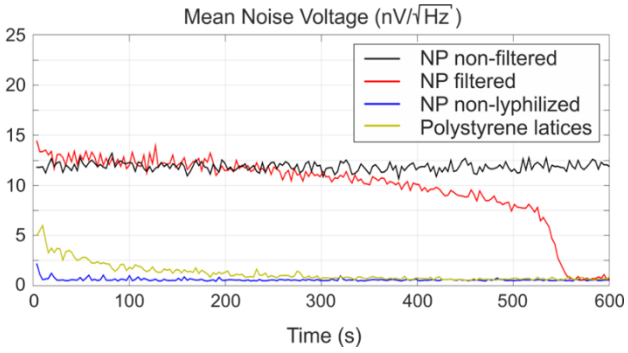

Figure 3: Mean noise voltage between 5 MHz and 6 MHz of the hydrophone output signal with respect to time. The cuvette contains filtered and non-filtered PLLA nanoparticles. NP non-lyophilized and Polystyrene beads show no cavitation activity, whereas both NP filtered and non-filtered do.

Fig. 3 shows that poly-(L)-lactic acid nanoparticles are acoustically active at 833 kHz and 1.5 MPa over a period of about 10 minutes.

4 Outlook

Presently our research team is planning a variation of methods in the preparation of nanoparticles and attachment of drugs to their surface. More acoustic measurements are planned to observe the different characteristics of the nanoparticles under different pressures and frequencies to evaluate the practicability and to test the potential of this method of treatment. Furthermore, the activity of cavitation is planned to be observed in tissue imitating material and real biological tissue.

Acknowledgment: The authors thank GE Healthcare for the contributed Optison®.

Author's Statement

Research funding: The Division of Pharmaceutics and the Division of Sensor Technology at the University of Erlangen funded this work. Conflict of interest: Authors state no conflict of interest. Informed consent: Informed consent is not applicable. Ethical approval: The conducted research is not related to either human or animals use.

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